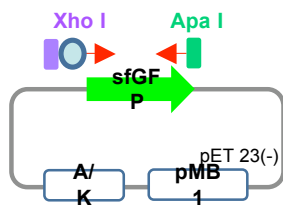


pUC19 [pBad/AraC - FliC-His – sfGFP(-7)]



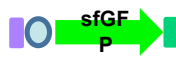
↓ PCR



↓ ゲル電チェック

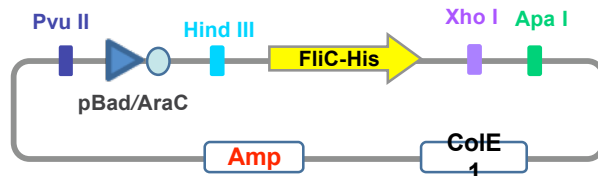
↓ Zymo

↓ Digestion
Xho I & Apa I

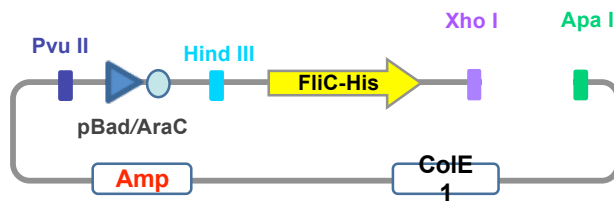


↓ ゲル抽出

↓ ゲル電濃度チェック



↓ Digestion
Xho I & Apa I



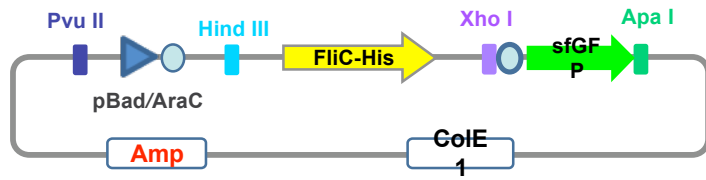
↓ ゲル抽出・ゲル電

↓ SAP

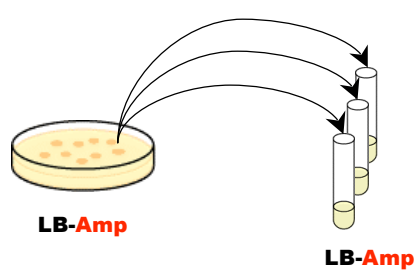
↓ Zymo

↓ ゲル電濃度チェック

↓ Ligation



↓ Incubation at 37°C, 12 h



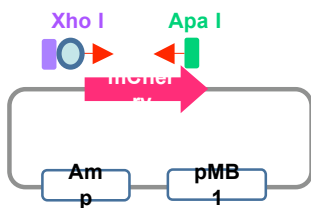
Incubation at 37°C, 12 h

↓ Phenotype
Check

↓ miniprep
↓ Digestion test
Xho I & Apa I

↓ Zymo
↓ Sequence 解析

pUC19 [pLac - FliC-Bio - mCherry]



↓ PCR



↓ ゲル電チェック

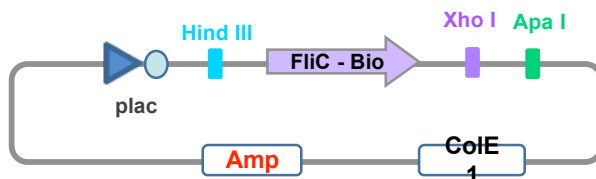
↓ Zymo

↓ Digestion
Xho I & Apa I

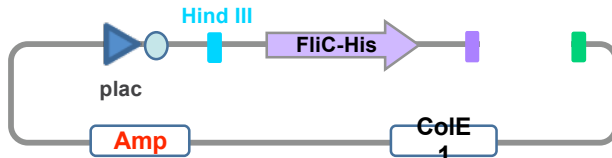


↓ ゲル抽出

↓ ゲル電濃度チェック



↓ Digestion
Xho I & Apa I



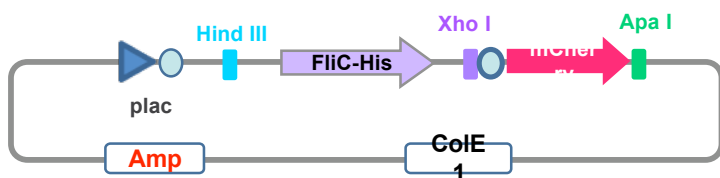
↓ ゲル抽出・ゲル電

↓ SAP

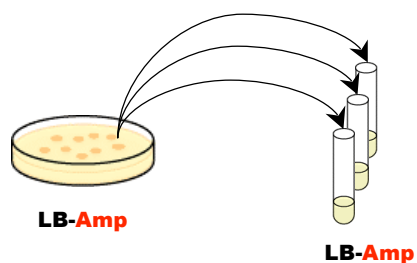
↓ Zymo

↓ ゲル電濃度チェック

↓ Ligation



↓ Incubation at 37°C, 12 h



Incubation at 37°C, 12 h

↓ Phenotype
Check

↓ miniprep

↓ Digestion test
Xho I & Apa I

↓ Zymo

↓ Sequence 解析

<PCR 条件>

95°C [5 min]
 ↓
 { 95°C [30 sec]
 ↓
 52°C [60 sec]
 ↓
 72°C [60 sec] } × 25
 ↓
 72°C [10 min]
 ↓
 6°C STORE

<PCR 混ぜ表>

template (100 ng)	1
primer F	5
primer R	5
dNTP	5
ThermoPol buffer	5
vent pol.	1
NFW	28
total	50 ul

<Digestion 条件>

① 25°C, 3 h
 ↓
 ② 37°C, 3 h

<Digestion 混ぜ表>

①	plasmid (100 ng/ul)	10
	NEB buffer #4	4
	BSA	1
	Apa I	1
	NFW	24
	total	40 ul
②	Xho I	1
	total	41 ul

<Ligation 条件>

25°C, 3 h

<Ligation 混ぜ表>

insert (100 ng/ul)	2
vector (100 ng/ul)	1
NEB ligation buffer	2
T4 DNA ligase	1
NFW	14
total	20 ul

<SAP 条件>

37°C, 1 h
 ↓
 65°C, 30 min

<SAP 混ぜ表>

vector (200-800 ng)	20
SAP buffer	5
SAP	1~2
NFW	23~24
total	50 ul